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Syndromes in suites of correlated traits suggest multiple mechanisms facilitating invasion in a plant range-expander

Lisa Johanna Tewes[1], Caroline Müller[1]

1 *Department of Chemical Ecology, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany*

Corresponding author: *Caroline Müller* (caroline.mueller@uni-bielefeld.de)

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Abstract

Various mechanisms can facilitate the success of plant invasions simultaneously, but may be difficult to disentangle. In the present study, plants of the range-expanding species *Bunias orientalis* from native, invasive and naturalised, not yet invasive populations were compared in a field common garden over two years. Plants were grown under two nitrate-regimes and multiple traits regarding growth, defence, antagonist loads and reproduction were measured. A rank-based clustering approach was used to assign correlated traits to distinct suites. These suites were analysed for "syndromes" that are expressed as a function of population origin and/or fertilisation treatment and might represent different invasion mechanisms. Indeed, distinct suites of traits were differentially affected by these factors. The results suggest that several pre-adaptation properties, such as certain growth characteristics and intraspecific chemical variation, as well as post-introduction adaptations to antagonists and resource availability in novel habitats, are candidate mechanisms that facilitate the success of invasive *B. orientalis* in parallel. It was concluded that rank-based clustering is a robust and expedient approach to integrate multiple traits for elucidating invasion syndromes within individual species. Studying a multitude of traits at different life-history and establishment stages of plants grown under distinct resource treatments reveals species-specific trade-offs and resource sinks and simplifies the interpretation of trait functions for the potential invasive success of plants.

Keywords

Glucosinolates, herbivory, invasion mechanisms, intraspecific variation, pathogens, nitrate allocation

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Introduction

To understand why plant species become dominant in novel habitats is one of the central aims of invasion ecology. Various pre-adaptations and post-introduction evolution events are considered as mechanisms for facilitating invasions in common hypotheses on this topic (Jeschke and Heger 2018). Pre-adaptations are traits that provide successful invaders with advantages over non-invasive congeners, turning them into ideal weeds (Baker 1965) in disturbed habitats (van Kleunen et al. 2015). Successful invaders show, for example, optimal resource-use efficiency or high environmental tolerance (Ren and Zhang 2009). High intraspecific variation in defensive compounds within and across populations may prevent (novel) antagonists adapting to plant chemistry, as proposed by the chemical diversity hypothesis (CDH; Wolf et al. 2011). Hypotheses on post-introduction evolution consider changes in traits in individuals after establishment in a non-native range. Many of these changes are assumed to be driven by the release from antagonists after migration (Keane and Crawley 2002, Mitchell and Power 2003). For example, the shifting defence hypothesis (SDH) postulates an increase in cheap toxic compounds but a decrease in expensive defences in plants growing in the novel range as a result of release from specialist enemies (Doorduin and Vrieling 2011). Facing distinct environmental conditions, (adapted) phenotypic plasticity in resource allocation to specific traits can also play a role in plant invasions, if the respective traits contribute to fitness (Richards et al. 2006, Davidson et al. 2011).

Several invasion hypotheses are substantively related but consider traits from different physiological contexts, whereas the choice of target traits for comparisons can be challenging. Moreover, species characteristics may be revealed in suites of multiple traits (Junker et al. 2017). Groups of plant species with trait co-variations or trade-offs within functional suites are often assigned into "syndromes" (Reich et al. 2003), typically regarding pollination (Fenster et al. 2004) or defence (Agrawal and Fishbein 2006, Travers-Martin and Müller 2008). For groups of invasive species, syndromes were found in certain trait characteristics (Schmidt et al. 2012) and defined as "associations of species biology and invasion dynamics with particular contexts" (e.g. establishment phase, habitat conditions) (Kueffer et al. 2013). However, also in individual invaders, multiple traits might be involved in adaptations to novel contexts (Zou et al. 2007). Thus, syndromes may also be expressed on the level of individual species and be defined as suites of correlated traits beneficial for, or altered by, invasion. Furthermore, different invasion mechanisms, potentially involving different suites of traits, can simultaneously occur in one species (Wolf et al. 2011, Guo et al. 2014) or act in a different life-history-stage (Ren and Zhang 2009) or establishment phase during range-expansion (Dietz and Edwards 2006). Surprisingly, comprehensive information on co-occurring mechanisms, which could be revealed from large datasets, is lacking for most invaders, including model species (Kueffer et al. 2013).

Clustering methods that structure datasets of multiple traits are commonly applied to determine behavioural dimensions and to test for consistency over time and within suites of correlated traits in animal individuals belonging to one species (Gyuris et al.

2011, Müller and Müller 2015, Blight et al. 2017). Such methods have recently been used to evaluate consistency in plant individuals within a species over time regarding various traits (Kuppler et al. 2016), but to the authors' knowledge, not yet to reveal suites of correlated traits and their "behaviour" in an invasion context.

In this study, associations were investigated between multiple traits in plants from different populations of a range-expanding perennial, *Bunias orientalis* L. (Brassicaceae), to reveal candidate mechanisms facilitating invasion. This species grows natively in Southeast Europe and Western Asia, but was introduced to Central Europe in the 18th century as a fodder plant and spread due to accidental human transport (Birnbaum 2006). In parts of Central Europe, the species is classified as invasive, rapidly establishing in disturbed, fertile dispersal corridors and spreading into adjacent habitats (Dietz et al. 1999, Birnbaum 2006). Thus, habitat fertilisation may facilitate the success of *B. orientalis* (Steinlein et al. 1996). In Western and Northern Europe, some populations can be considered as naturalised but not yet invasive following the nomenclature of Richardson et al. (2000), i.e. they do not form dominant stands far from parental plants (Harvey et al. 2010, Fortuna et al. 2014). As defence metabolites, *B. orientalis* produces several glucosinolates (Tewes et al. 2018), which are characteristic for Brassicales and serve important roles in (novel) biotic interactions (Müller 2009). Thus, glucosinolates are valuable target compounds to evaluate relationships between defence and invasion in this species.

Plants were grown from populations of native, invasive and naturalised status over two years in a field common garden in an area where *B. orientalis* does not occur. Plants were exposed to two nitrate-fertiliser treatments to investigate the influence of resource availability on various traits potentially facilitating successful establishment, regarding growth, defence, antagonist loads and reproduction. Suites of correlated traits were determined and it was hypothesised differential influences of population status and/or fertilisation on traits clustered in distinct consistent suites. When significantly affected by one of these factors, these suites were considered to express syndromes that might represent distinct invasion mechanisms. Furthermore, it was expected that plants from invasive populations reveal differential mechanisms that characterise those plants as more successful colonisers.

Methods

Plant material and common garden design

Silicles of the perennial plant *B. orientalis* were collected from 12 populations of the native, invasive and naturalised distribution range, following the status assignment of the regions by Harvey et al. (2010) (Table 1). Thus, populations from Russia (AL), Turkey (T3, T4) and Romania (RO) were considered as 'native', populations from Lithuania (LT), the Czech Republic (CB) and Germany (JE, WU) as 'invasive' and populations from The Netherlands (DR, DI) and France (GO, PA) as 'naturalised' (Table 1). From seven populations, a F1 generation produced in the preceding year was used (for details see Suppl. material 1). From the remaining populations (native: RO;

Table 1. Origin, invasion status and survival of *Bunias orientalis* populations. Populations were grouped by status according to their ecology, plant frequency or distribution in the origin region (Harvey et al. 2010; Tewes et al. 2018), following the nomenclature of Richardson et al. (2000). The number of plants that survived the winter between 2015 and 2016 are given in relation to the original number.

Code	City/region	Country	Latitude	Longitude	Status	Survival
AL	Altai	Russia	50°49.17'N†	86°16.41'E†	native	10/12
T3	near Rize	Turkey	40°43.97'N	40°47.41'E	native	12/12
T4	near Rize	Turkey	40°44.33'N	40°44.12'E	native	10/12
RO	Iaşi	Romania	47°11.24'N	27°33.44'E	native	10/12
LT	*Mixed*‡	Lithuania	54°54'N‡	23°56'E‡	invasive	12/12
CB	Beroun-Zavadilka	Czech Republic	49°56.57'N	14°4.08'E	invasive	12/12
JE	Jena	Germany	50°52.42'N	11°35.76'E	invasive	11/12
WU	Würzburg	Germany	49°50.95'N	9°51.94'E	invasive	10/12
DR	Drempt	The Netherlands	52°0.39'N	6°9.62'E	naturalised*	12/12
DI	Driel	The Netherlands	51°58.07'N	5°51.17'E	naturalised*	9/12
GO	Gondreville	France	48°41.23'N†	5°57.9'E†	naturalised*	12/12
PA	Pasques	France	47°21.98'N†	4°51.36'E†	naturalised*	11/12

† coordinates estimated from origin information

‡ coordinates averaged from four origin populations from which seeds were pooled

* named "exotic" in Harvey et al. (2010) and Tewes et al. (2018)

invasive: LT, CB; naturalised: GO, PA), seeds only became available in 2014 and were thus used directly for the experiments. Thus, maternal effects influencing the traits of certain populations cannot be excluded. However, plants were grown and monitored for two years and, because at least half of the populations per status were available as F1-generation, a low impact on the statistical data analyses was assumed. Accordingly, none of the results of the study suggested evidence for an interfering influence of the seed generations of populations on the traits observed.

In April 2015, 30 seeds per population were sown in individual 50-mL pots on seedling soil (Archut Fruhstorfer Erde Typ LAT-Terra Standard Pickiererde; Hawita, Vechta, Germany) and kept in a greenhouse (14:10 h day:night, 15–20 °C). After three weeks, 15 seedlings per population were transferred to 2-L pots (11.3 × 11.3 × 21.5 cm) with poorly fertilised soil (C 710 with Cocopor, Stender, Schermbeck, Germany). The plant pots were arranged in 15 plots, each containing one plant per population, and were watered three times per week. Three weeks after re-potting, seven plots were assigned to a low and seven to a high fertilisation treatment to test for plant responses to nitrate availability. One additional plot received intermediate fertilisation. Each plant was fertilised by adding 50 mL of a mineral nutrient solution (modified after Hoagland and Arnon 1950) to the pot containing 1 mM (low), 4 mM (high) or 2 mM (intermediate) $Ca(NO_3)_2$ (for other constituents see Suppl. material 1). The volume was increased to 100 mL per pot after three weeks and the fertilisation treatment repeated once per week over the entire experimental period, with a break from October to early April.

Three weeks after start of the fertilisation (June 2015), all pots were transferred to a field common garden near Bielefeld University (Germany; latitude: 52°2.022'N,

longitude: 8°29.718'E; 146 m a.s.l.). A total of 144 plants were arranged in 12 plots, each containing one plant per population with a random position within each plot (in total n = 6 plants per fertilisation treatment and population; for detailed experimental set-up see Suppl. material 2, fig. S1). The pots were buried about 19 cm deep in the ground. To eventually replace a few of the experimental plants that died during the experiment, a surplus of one plot per fertilisation treatment and one plot with plants of the intermediate fertilisation treatment (n = 12 plants per plot) were set up next to the experimental area.

The experimental area was located in North-Western Germany, where *B. orientalis* does not occur in the wild (Harvey et al. 2010, Tewes LJ pers. obs.). Therefore, the present antagonists can be considered as naïve in interaction with this species. The area had been ploughed two months before the experimental plants were set up. Over the two years, the experimental plants were increasingly surrounded by naturally occurring vegetation, comprising associative character species of the Molinio-Arrhenatheretea (i.e. cultivated grassland communities, after Oberdorfer 1990), but only a low number of other Brassicaceae. Slug pellets were regularly spread to exclude slugs as these non-selective herbivores were highly abundant.

Measurements of growth, antagonist load and reproduction traits

After five weeks of plant acclimatisation in the common garden, several traits regarding antagonist load were measured. Insect observations were repeated for every experimental plant ten times within six weeks until late August. Therefore, plants were first carefully approached to count and identify escaping insects and, afterwards, the leaves were searched for eggs, larvae and adults of herbivorous and predatory insects. Each observation was made between 1300 h and 1600 h on two consecutive days for all plots. Identifications were made on the family-level and related species recorded as morphotypes. As insect occurrence on *B. orientalis* was overall very low, insect count data were summed for every plant over the ten observations. Thus, insect counts might be biased by repeated counts of individuals, especially of immobile insects. However, a constant presence on a plant represents acceptance of, or even reproduction on, that plant.

During the last observation in the first year, plant damage from chewing-biting insects was monitored by estimating the consumed leaf area per plant using templates of various sizes. Leaf mines of identical morphs and infestation spots of (likely fungal) pathogens were counted. Furthermore, the numbers of rosette and offshoot leaves were counted and the length was measured of the longest leaf per plant (i.e. rosette expansion) as growth traits. Finally, ten leaf discs (12.7 mm diameter) were taken from the third youngest leaf pair per plant which showed no obvious visual damage, immediately frozen in liquid nitrogen and stored at –80 °C for later analysis of defence traits (see below).

Early in the second year (April 2016), 10 of the 13 dead plants were replaced with plants of the same fertilisation treatment, where possible. All except three plants (from three populations) produced stems with small leaves from their rosettes and

were scored daily for the first flower opening between early May and late June. In early July, rosette, offshoot and stem leaves as well as pathogen infestation spots per plant were counted. Due to the greatly differing plant growth form (i.e. number, type and size of leaves), comparable insect observations were not possible. Between late July and mid August, the reproductive output was measured as the number of silicles per plant. As reproduction-related growth traits, the stems and branches were counted and the lengths of the highest stem and the longest branch were measured. The experiment concluded at the end of August 2016.

Analyses of leaf mass per area and glucosinolates as defence traits

Leaf discs harvested in 2015 were lyophilised and weighed to calculate the leaf mass per area (LMA) as a mechanical defence trait. From the same leaf material, glucosinolates were analysed after conversion to desulphoglucosinolates using high performance liquid chromatography, as in Martin and Müller (2007). Desulphoglucosinolates were identified based on their UV spectra, retention time and verification by LC-MS (Fortuna et al. 2014). For quantification, the peaks were integrated at 229 nm; thereby response factors were considered for different side chain classes (González-Megías and Müller 2010).

Data analysis

Statistical analyses were done with R (version 3.0.3; R Core Team 2014). To evaluate the diversity of leaf glucosinolates and of herbivorous insect morphs occurring on each plant, the Shannon index [$H_S = - \Sigma p_i * \ln p_i$] (Shannon and Weaver 1949) (package *vegan*) was calculated, where p is the glucosinolate concentration or herbivore morph abundance. Linear mixed-effects models (LMMs) (package *lme4*) were computed to test for effects of population status (native, invasive, naturalised), fertilisation treatment (low, high) and their interaction (fixed factors) on selected plant traits (Table 2). Within these models plant population and plot identity of individuals were included as random factors. LMMs were calculated on the raw data, log-transformed or square root-transformed data (for details on model selection see Suppl. material 1). The finally-used transformations of the traits are given in Table 2. All models were fitted with a maximum likelihood approach, where *P* values were computed based on likelihood ratio tests (Chi^2 tests). The residuals of all final models were inspected for normality (visually; Shapiro-Wilk test) and homoscedasticity (Levene test; package *car*) and did not show obvious deviations from these assumptions.

To test for correlations between traits, pairwise Spearman rank correlation tests were applied on the untransformed dataset. Therefore, a correlation matrix of Spearman's *rho* and the corresponding *P* values using the 'rcorr' function (package *Hmisc*) were generated. To find potential associations between traits, an agglomerative cluster analysis (unweighted pair-group arithmetic average method, UPGMA) was performed using

Table 2. Statistics for traits of *Bunias orientalis* plants. Populations of different status (native, invasive, naturalised, n = 4 populations each) were grown in a field common garden under two fertilisation treatments (low, high) (n = 5–6 per population and treatment). Traits were analysed using linear mixed-effect models (maximum likelihood approach, Chi² likelihood ratio tests) with status, fertilisation treatment and their interaction as fixed effects and population and common garden plot identity as random effects. Traits were grouped based on a cluster analysis of Spearman rank-correlations (Suite) and group ranks re-analysed with plant individual as an additional random factor. Transformations: a: no transformation; b: log-transformation; c: square root-transformation; +1: 1 added to whole dataset. P values < 0.1 and > 0.05 in bold and italic, P values < 0.05 in bold. no.: number; GS: glucosinolate; conc.: concentration.

		Status		**Fertilisation**		**Status×Fertilisation**	
	Suite	$Chi^2_{2\,df}$	P	$Chi^2_{1\,df}$	P	$Chi^2_{2\,df}$	P
First year							
Growth							
Total leaf no.[b]	A	4.69	***0.096***	7.18	**0.007**	2.01	0.366
Length longest leaf[a]	B	4.46	0.108	4.79	**0.029**	0.02	0.992
Defence							
Leaf mass per area[b]	C	5.69	***0.058***	0.38	0.537	0.06	0.973
Total GS conc.[c]	C	4.54	0.103	1.46	0.227	1.98	0.372
GS diversity[a]	C	2.50	0.286	0.40	0.528	3.40	0.183
Antagonist load							
Pathogen spot no.[b]	B	6.64	**0.036**	0.40	0.526	0.83	0.661
Herbivore no.[c]	B	4.00	0.135	0.21	0.651	1.82	0.404
Herbivore diversity[a]	A	2.80	0.247	1.01	0.316	1.87	0.394
Leaf herbivory[b+1]	B	2.45	0.294	2.18	0.140	1.73	0.420
Beneficial Insects							
Predator no.[c]	B	3.21	0.201	1.22	0.269	0.53	0.769
Second year							
Growth							
Total leaf no.[c]	D	7.57	**0.023**	0.11	0.740	3.72	0.156
Antagonists							
Pathogen spot no.[b+1]	D	2.47	0.291	0.06	0.802	4.57	0.102
Reproduction							
Flowering delay[c]	C	0.66	0.720	3.50	***0.061***	5.24	***0.073***
Length highest stem[a]	D	4.75	***0.093***	1.23	0.267	0.59	0.743
Silicle no.[b]	D	5.96	***0.051***	0.22	0.638	5.23	***0.073***
Rank-based clusters							
	n						
Suite A[a]	12	3.70	0.158	5.27	**0.022**	2.48	0.290
Suite B[a]	30	7.20	**0.027**	3.34	***0.068***	0.10	0.953
Suite C[a]	20–24	3.28	0.194	0.49	0.485	1.14	0.565
Suite D[a]	18–24	4.68	***0.096***	0.11	0.743	6.07	**0.048**

the 'agnes' function (package *cluster*) on a matrix of 1 minus the absolute Spearman's *rho* values as rank-based distance measures. The actual number of groups within the dendrogram clusters was revealed based on the highest average silhouette width found in multiple silhouette plots generated, assuming different numbers of groups (package *cluster*).

Consistency was tested across traits within the cluster-groups (suites) and across the entire cluster using Kendall's coefficient of concordance *W* (package *irr*). As the corresponding significance test cannot handle negative correlations within groups, the ranks of one trait (LMA) were manually reversed. For one group consisting of only two traits, a pairwise Spearman rank correlation was used. To test if the suites of correlated traits reveal different syndromes, the raw data ranks were combined for individuals of all traits in each group separately and these datasets analysed using LMMs as described above. Therefore, plant identity was used as an additional random factor to control for multiple measures with individual plants and each incomplete dataset was reduced to individuals, in which at least two traits were measured.

Data resources

The data underpinning the analyses reported in this paper are deposited in the Dryad Data Repository at https://doi.org/10.5061/dryad.v17p8m4.

Results

Growth

The total number of leaves differed depending on the population status, being lower in naturalised than in invasive and native populations (Fig. 1a, b; for individual leaf types see Suppl. material 3, fig. S2). The population status influenced the leaf numbers by trend in the first year and affected them significantly in the second year (Table 2). The length of the longest leaf per plant (first year) did not differ significantly according to status (Table 2) but showed population-specific differences, whereby plants of the native populations T3 and T4 had shorter leaves than all other plants (Fig. 1c). The total number of leaves and the length of the longest leaf were significantly higher in highly fertilised plants in the first year (Table 2, Fig. 1a, c). In contrast, in the second year, the total leaf number was neither significantly affected by fertilisation nor by population status (Table 2; Fig. 1b).

Defence

The LMA (first year) tended to be influenced by the population status (Table 2), being higher in plants of naturalised populations than in those of native and invasive ones (Fig. 1d). Glucosinolate concentrations and diversity were neither affected by plant origin nor fertilisation (Table 2), but strongly varied within and between populations (Fig. 1e, f).

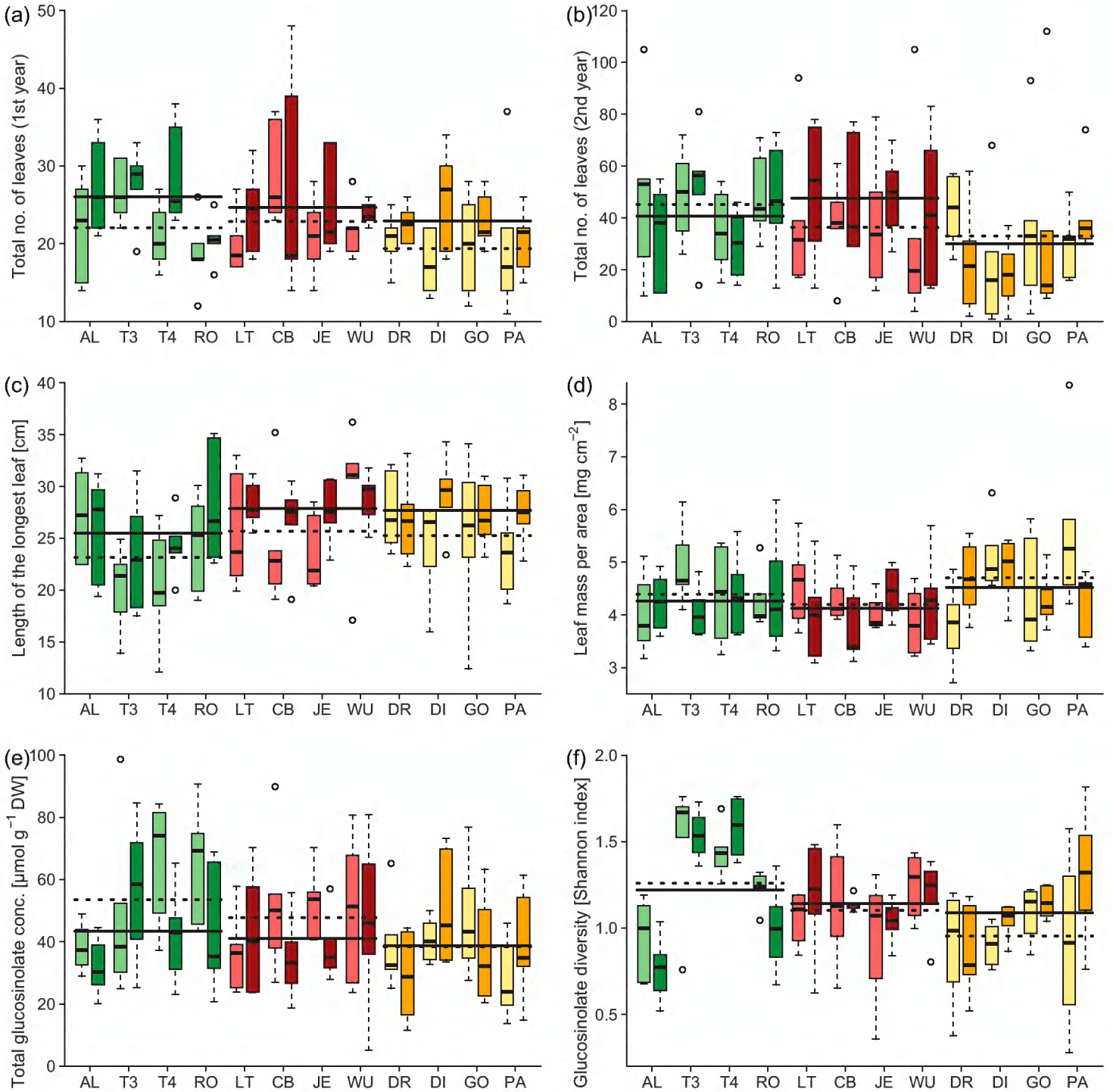
(a)
Total no. of leaves (1st year)
(b)
Total no. of leaves (2nd year)
(c)
Length of the longest leaf [cm]
(d)
Leaf mass per area [mg cm^{-2}]
(e)
Total glucosinolate conc. [µmol g^{-1} DW]
(f)
Glucosinolate diversity [Shannon index]
AL T3 T4 RO LT CB JE WU DR DI GO PA

Figure 1. Growth and defence traits of *Bunias orientalis* plants. Twelve populations of native (green), invasive (red) or naturalised (yellow) status were grown in a field common garden under two nitrate-fertilisation treatments, low (light shade) and high (dark shade) (n = 6 per population and fertilisation treatment, n = 5–6 in (**b**); for population codes see Table 1). **a** Total number of leaves per plant in the first year (rosette and offshoot leaves) and **b** in the second year (rosette, offshoot and stem leaves) **c** length of the longest leaf, **d** leaf mass per area, **e** total leaf glucosinolate concentration and **f** Shannon diversity index for nine glucosinolates in the first year. Box plots: median, 5th, 25th, 75th, 95th percentiles; open dots: outliers. Lines along four populations of identical status: status mean values for low (dashed) and high (solid) fertilisation treatments.

Antagonist loads

The number of pathogen infestation spots in the first year was significantly influenced by the population status, being on average lowest in plants of native and highest in those of invasive populations (Table 2, Fig. 2a). In the second year, no such differ-

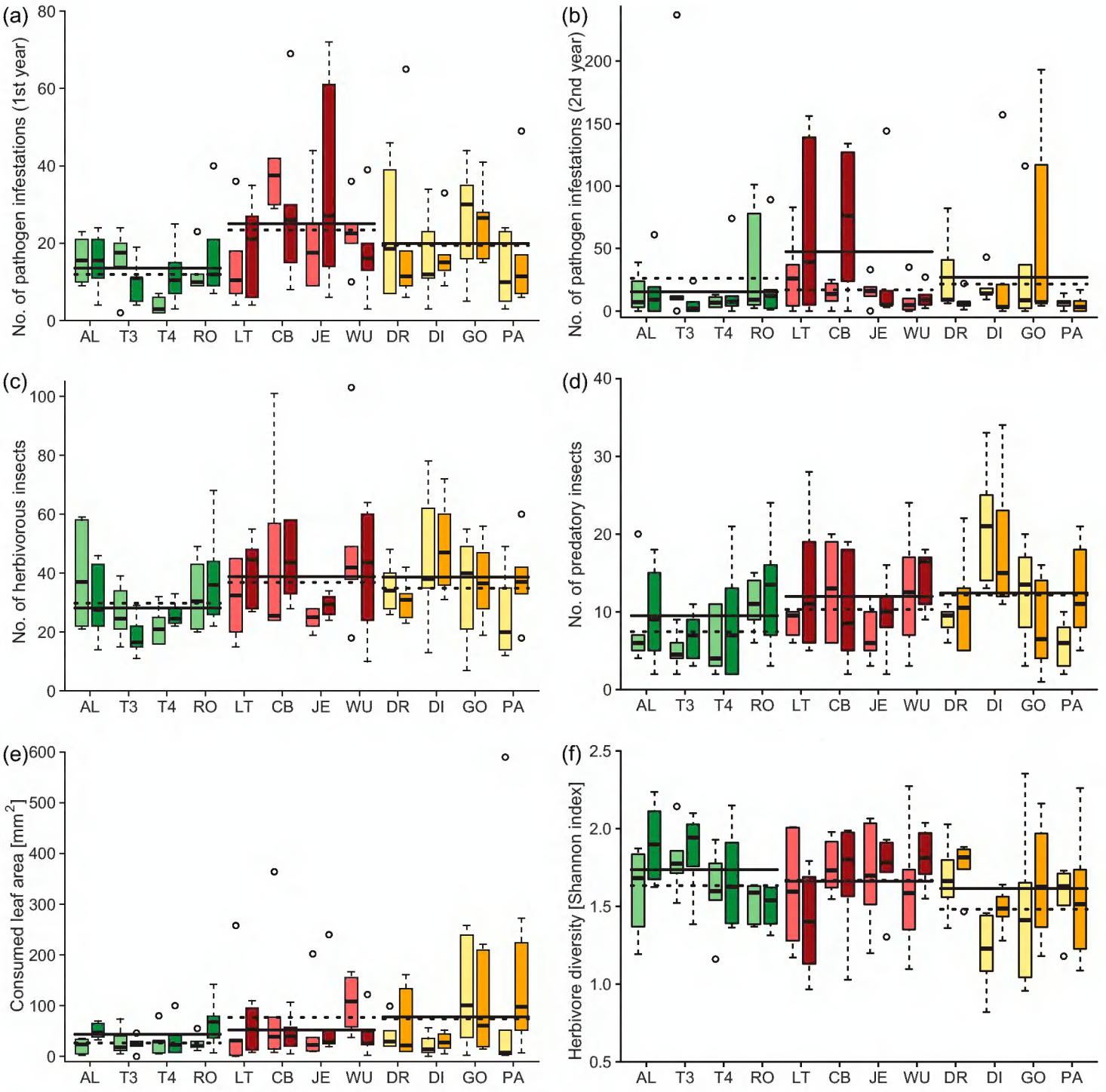

Figure 2. Antagonist loads of *Bunias orientalis* plants. Twelve populations of native (green), invasive (red) or naturalised (yellow) status were grown in a field common garden under two nitrate-fertilisation treatments, low (light shade) and high (dark shade) (n = 6 per population and fertilisation treatment, n = 5–6 in (**b**); for population codes see Table 1). **a** Pathogen infestation spots in the first year and **b** in the second year, **c** cumulative total number of herbivorous and **d** predatory insect morphotypes, **e** leaf area per plant consumed by herbivores and **f** Shannon diversity index for the abundance of 24 insect morphotypes in the first year. Box plots: median, 5th, 25th, 75th, 95th percentiles; open dots: outliers. Lines along four populations of similar status: status mean values for low (dashed) and high (solid) fertilisation treatments.

ence was found (Table 2), but individuals of some populations were highly infested (Fig. 2b). Overall, 24 herbivorous insect morphs were found on the plants in the first year, comprising chewing-biting, piercing-sucking and leaf mining herbivores (Suppl. material 4, fig. S3) whose numbers were overall correlated (Suppl. material 6, fig. S5). Moreover, eight predator morphs were observed. Both the number of herbivorous and

predatory insects were neither significantly influenced by population status nor fertilisation treatment (Table 2; Fig. 2c, d). Herbivore diversity and damage also showed no significant differences between plants of different population status or fertilisation (Table 2), but differed between populations (Fig. 2e, f).

Reproduction

In the second year, highly fertilised plants tended to flower earlier, particularly those of invasive populations (Table 2, Fig. 3a). The height of the flowering stems was by trend influenced by the population status (Table 2); plants from native populations had shorter stems than those of non-native origin (Fig. 3b). The number of silicles per plant was nearly significantly influenced by population status and the interaction between status and fertilisation (Table 2), with most silicles in highly fertilised plants of invasive and fewest in those of native populations (Fig. 3c).

Suites of correlated traits and syndromes

The agglomerative cluster analysis of selected plant trait ranks resulted in four suites of consistently correlated traits, A–D (Fig. 4a, b). Additionally, several traits correlated across distinct suites (Fig. 4a, b), as highlighted in the discussion. Suite A comprised the total number of leaves and herbivore diversity in the first year, being significantly correlated ($rho = 0.314$, $P < 0.001$; Fig. 4a). The combined ranks from this suite revealed a significantly positive fertilisation effect, but there was no influence of plant origin (Table 2; Fig. 4c). Within suite B ($W = 0.442$, $P < 0.001$), the length of the longest leaf was positively correlated with the total numbers of pathogen infestation spots, herbivores and predators and with herbivore damage per plant (Fig. 4a, b). For the combined ranks of traits in suite B, fertilisation tended to have a positive effect and a significant influence of the population status was found (Table 2), with the combined ranks being lowest in plants of native populations (Fig. 4c). Suite C ($W = 0.395$, $P < 0.001$) consisted of chemical defence traits, the LMA and the flowering start date and was the only suite comprising correlated traits for both years (Fig. 4b). The combined ranks of traits in this suite were neither affected by population status nor fertilisation (Table 2, Fig. 4c). Interestingly, the LMA was the only trait revealing consistently negative correlations with other traits, especially with the total glucosinolate concentration within suite C (Fig. 4a, Suppl. material 6, fig. S5). Suite D ($W = 0.464$, $P < 0.001$) covered all remaining traits measured in the second year, comprising growth, pathogen load, reproduction-related growth and reproductive output which were nearly all correlated (Fig. 4a, b). The combined ranks of traits within suite D revealed a significant interaction of both fixed factors, as well as by trend a status effect (Table 2); at high fertilisation, ranks were higher in

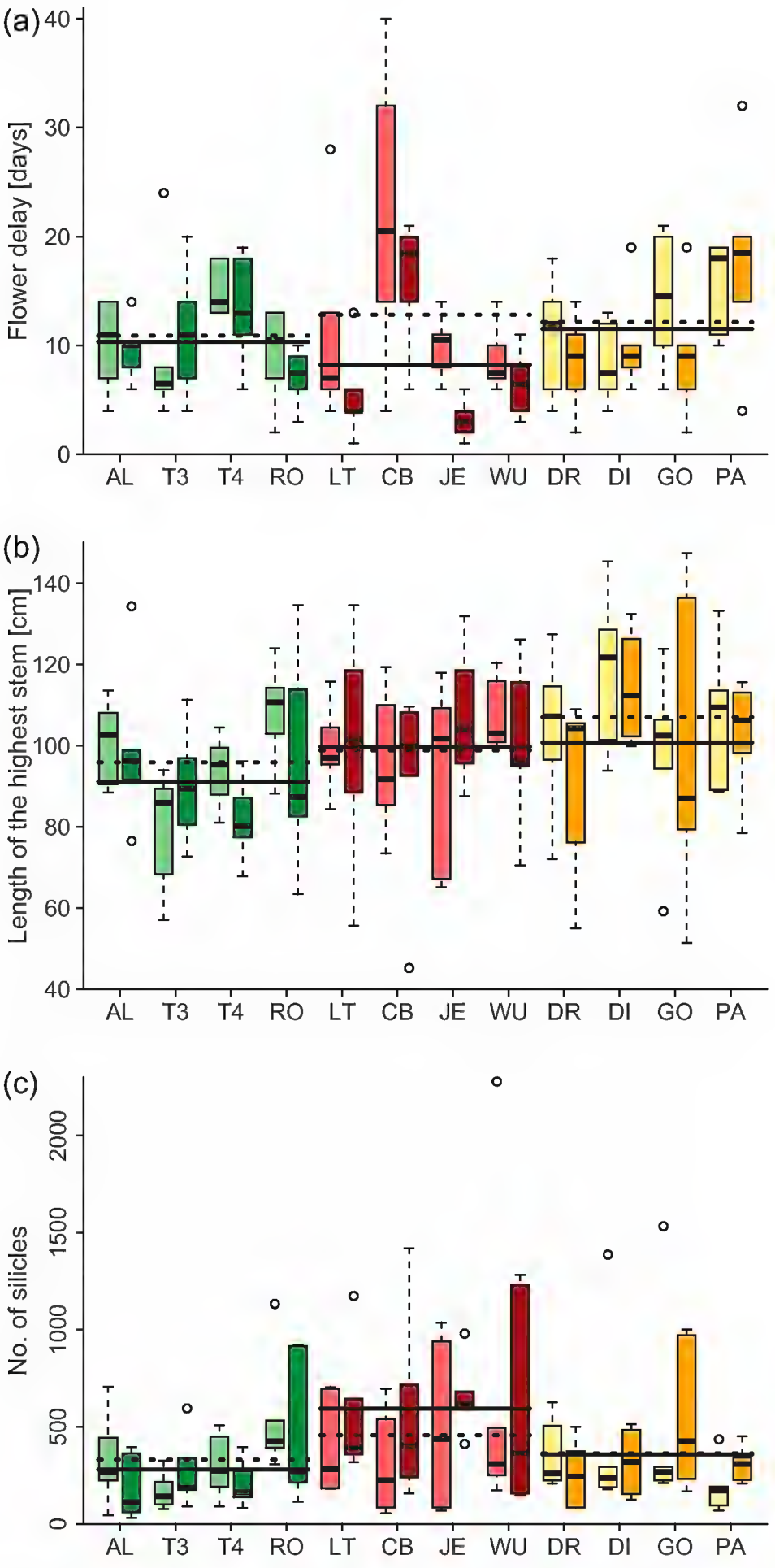

Figure 3. Reproduction traits of *Bunias orientalis* plants. Twelve populations of native (green), invasive (red) or naturalised (yellow) status were grown in a field common garden under two nitrate-fertilisation treatments, low (light shade) and high (dark shade) (n = 4–6 per population and fertilisation treatment; for population codes see Table 1). **a** Flowering start date, **b** height of the longest stem with inflorescences and **c** number of developed silicles. Box plots: median, 5th, 25th, 75th, 95th percentiles; open dots: outliers. Lines along four populations of similar status: status mean values for low (dashed) and high (solid) fertilisation treatments.

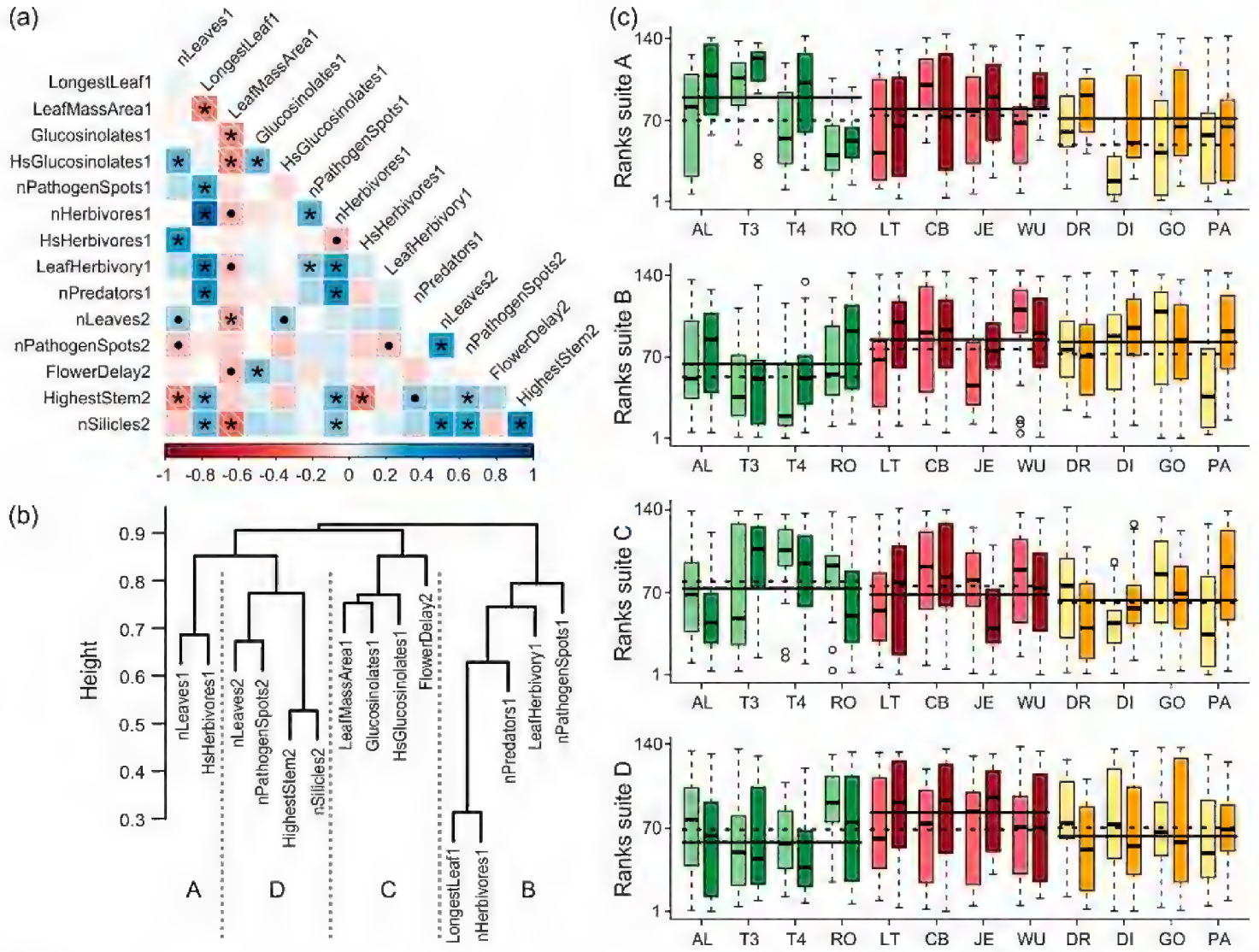

Figure 4. Rank-based correlations and clustering amongst individual traits of *Bunias orientalis* plants. Twelve populations were grown in a field common garden over two years. **a** Pairwise correlations of traits with heatmap shadings based on Spearman's *rho* (n = 131–144 individuals in pairwise comparisons). Correlations are marked as positive (blue) or negative (red, hatched fields). Asterisks: significant correlations, $P < 0.05$; dots: tendencies, $P < 0.1$. **b** Agglomerative cluster analysis (coefficient 0.3) of traits based on 1 minus Spearman's *rho* values as pairwise distances (UPGMA method). Dashed lines divide four suites of consistently correlated traits (Spearman's *rho*; Kendall's W, each $P < 0.001$), identified using silhouette plots (not shown). LeafMassArea: leaf mass per area; Hs: diversity of (Shannon index); 1, 2: year the trait was obtained. **c** Combined ranks of the four suites from (**b**), displayed over 12 populations of native (green), invasive (red) or naturalised (yellow) status grown under two fertilisation treatments, low (light shade) and high (dark shade); for population codes see Table 1. From top to bottom: suite A (n = 12 per population and fertilisation treatment), B (n = 30), C (n = 20–24) and D (n = 18–24). Box plots: median, 5th, 25th, 75th, 95th percentiles; open dots: outliers. Lines along four populations of identical status: status mean values for low (dashed) and high (solid) fertilisation treatments.

invasive plants and, in contrast, lower in plants from native and naturalised populations (Fig. 4c).

However, in all models, most of the variation was explained by the plant individual as a random factor (not shown), demonstrating high overall consistency within individuals. Accordingly, an overall consistency in individuals across all traits was revealed (W = 0.148, $P < 0.001$), although the W value was much lower than for trait ranks within groups.

Discussion

This study revealed four suites of correlated traits in *B. orientalis*, which were differentially affected by the invasion status of populations and nitrate-fertiliser supply. The resulting syndromes may be considered as alternative strategies for successful establishment. The response of the individual traits is discussed within the syndromes to which they contributed.

Resource allocation and leaf production in the first year

As syndrome in suite A, vigorous leaf production of *B. orientalis* was enhanced by fertilisation and associated with high herbivore diversity. As high herbivore diversity overall corresponded to low infestation by chewing-biting herbivores (Suppl. material 6, fig. S5), high fertilisation may enhance plant resistance in this species. For example, a high leaf number also correlated with high glucosinolate diversity (Fig. 4a), which can have a negative influence on caterpillar performance (Tewes et al. 2018). An effective resource allocation to leaf growth, as also found by Steinlein et al. (1996), may represent a feature of *B. orientalis* that allows the species to exploit the resources of new (disturbed) habitats, when introduced. Moreover, *B. orientalis* possesses further traits that characterise a pre-adapted ideal weed as defined by Baker (1965). For example, it can re-grow vegetatively from small root-fragments and is cross-pollinated but it is also self-compatible, with high production of long-lived seeds (Birnbaum 2006).

Longer leaves and higher antagonist loads in non-native plants

The syndrome of the traits in suite B suggests that plants of non-native populations were more attractive than those of native populations to generalist antagonists, especially pathogens. However, they may tolerate the overall moderate antagonist attacks in non-native habitats, for example, by expansive rosettes, offering putative advantages in plant competition. In contrast, plants of native populations produced many leaves with high resistance, indicated by a low number of pathogen infestation spots on those plants. The infestation by fungal pathogens mainly depends on physico-chemical characteristics of the plant surface (Müller and Riederer 2005) and leaf secondary metabolites (Grayer and Harborne 1994) and can thus be regarded as a powerful indicator for intraspecific changes in leaf defence. Susceptibility to damage from (root) pathogens in the novel range is considered important in plant invasions (van der Putten 2002) and several studies found higher resistance to pathogen infestation in invasive compared to native species (Agrawal et al. 2005, Han et al. 2008). However, intraspecific post-introduction alterations in plant resistance to (fungal) pathogens, as indicated here, have largely been neglected (but see Wolfe et al. 2004).

Interestingly, glucosinolates appeared not to be involved in the proposed changes in resistance upon invasion, as they clustered in another suite. However, higher concentrations of other low-cost toxic compounds might largely prevent non-native *B. orientalis* plants from antagonist attack, while some costly defences seemed to be decreased, as proposed by the SDH (Doorduin and Vrieling 2011). Indeed, a predominance of trichomes, which represent an effective mechanical barrier against antagonists (Handley et al. 2005), as well as digestibility-reducing defences (trypsin proteinase inhibitors) characterise *B. orientalis* compared to other Brassicaceae species (Travers-Martin and Müller 2008). These presumably costly defences may act as a resource sink and may potentially be reduced as a result of enemy release. Accordingly, a lower number of branched trichomes has been found in plants from non-native populations compared to native *B. orientalis* from Turkey (Fortuna et al. 2014), which were also characterised by low antagonist loads in the present study (Fig. 4a, c and e).

Finally, a strong correlation between herbivore and predator counts suggests that predators such as *Chrysoperla carnea* (Diptera: Syrphidae), *Episyrphus balteatus* (Neuroptera: Chrysopidae) and ichneumonids effectively controlled the herbivores on *B. orientalis*. These interactions may be partly mediated by plant volatiles that can attract these organisms (Kessler and Baldwin 2001). The complexity of the food-web structure rather than individual species ultimately determine the herbivore impact on a plant and the success of invasive species (Smith-Ramesh et al. 2017).

Defence and flowering start trade-offs and intra-population variation

Suite C combined defence by glucosinolates trading-off with defence by LMA and early flowering in the second year, which did not differ between plants of different status and fertilisation. A slightly higher LMA in plants of naturalised populations (Fig. 1d) presumably made these plants harder to consume. Indeed, a negative correlation of LMA with the number of chewing-biting herbivores suggests a contribution of this trait to defence in *B. orientalis*. Moreover, the elevated defence by LMA might partly compensate for lower performance in growth and reproduction in individual plants, as indicated by negative correlations with such traits (Fig. 4a).

The high variation in glucosinolate concentration and diversity within populations found in the present field experiment is in accordance with an earlier study including the same *B. orientalis* populations kept under laboratory conditions (Tewes et al. 2018). This high within-population variation in chemical defence concentration and composition may impede antagonist adaptations, as predicted by the CDH (Wolf et al. 2011). Moreover, a trade-off between glucosinolate concentration and LMA was revealed. A shift between chemical and mechanical defence by LMA likewise occurs in some milkweed species (Agrawal and Fishbein 2006). Thus, the CDH may be extended to comprise variability in both chemical and mechanical defences. This study underlines the importance of regarding intraspecific variation to understand effects on plant-associated communities, as was also suggested by Kuppler et al. (2016).

The combination of high glucosinolate concentrations and late flowering, revealed in this study, has likewise been found in *Brassica rapa* in response to stress (Steinbrenner et al. 2012) and might thus present a physiological trade-off. Assuming comparable glucosinolate concentrations in the overwintering plant parts, plants with high glucosinolate concentrations might delay resource allocation in reproduction without running the risk of being consumed before flowering starts. Intriguingly, early flowering of highly fertilised *B. orientalis* plants of invasive populations indicates effective resource allocation to reproduction in those plants, as was also revealed by other reproduction-related traits clustering in suite D.

Effective resource allocation in invasive plants in the second year

Suite D suggests that the full potential of invasive *B. orientalis* as successful colonisers is revealed in the second reproductive year, in which well-fertilised soils are effectively exploited, maximising plant performance. Particularly, highly fertilised plants of invasive populations stood out by, on average, high silicle and leaf numbers (Figs 1b, 3c) and high values in reproduction-related growth traits (Suppl. material 5, fig. S4). This finding is a strong indication for adapted phenotypic plasticity in nitrate allocation to reproduction in invasive populations, which would need to be tested by using clones, full- or half sibs (Richards et al. 2006). Strikingly, at low fertilisation, the ranks in suite D were similar amongst all plants. Thus, fertiliser treatments allow resource sinks to be identified and to make predictions about the contribution of nutrient availability in invaded habitats to plant syndromes relevant for successful colonisation. In contrast to the invasives, plants of native populations invested their resources in the second year mainly in vegetative reproduction with offshoots, which is not necessarily a fitness disadvantage (Davidson et al. 2011). Moreover, putative increased defences produced in native plants in the first year might persist in the second year and might be more important than high propagule pressure in co-evolved habitats.

Interestingly, plants of naturalised populations differed from the invasive ones in essential traits within this suite, highlighting the importance of examining interactions of non-native species and their environment in different establishment stages (Kueffer et al. 2013). As naturalised populations are potentially more isolated, low exchange between individuals might result in genetic depletion (Nei et al. 1975). Genetic diversity probably plays an important role in ecological and evolutionary processes (Hughes et al. 2008), but its contribution to invasion is complex and still not fully understood (Dlugosch et al. 2015). In *B. orientalis,* genetic diversity tended to be reduced in naturalised (= exotic) populations compared to native and invasive ones (Tewes et al. 2018), which might somehow be related to the reduced performance of these populations in the present study. If these naturalised populations become connected by more frequent introductions or establishments in the future, they might reveal a colonisation potential comparable to the invasive plants.

Conclusions

It could be demonstrated that regarding differential suites of traits within one invasive species, a variety of pre-adaptations and post-introduction evolution mechanisms, potentially beneficial for invasion, was revealed. Forming rank-based suites of functional traits over the life-history is a promising, integrative approach to identify syndromes displaying potential invasion strategies within species. More research on multiple mechanisms (and their interactions) facilitating individual migrating species in parallel should enhance the mechanistic understanding of novel ecological pattern. Thereby, a high number of traits should be monitored to discover species-specific trade-offs and to avoid misinterpretation of single trait functions. Finally, specifically in studies, in which bi- or perennial species are investigated, approaches that capture traits from different life-history stages are highly recommended.

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Supplementary material 1

Methods S1: Details for plant treatment and data analysis
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Data type: method
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Supplementary material 2

Figure S1. Design of the common garden field experiment
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Data type: image
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Supplementary material 3

Figure S2. Growth traits of *Bunias orientalis* plants
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Supplementary material 4

Figure S3. Herbivore loads of *Bunias orientalis* plants
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Supplementary material 5

Figure S4. Reproduction traits of *Bunias orientalis* plants
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Supplementary material 6

Figure S5. Pairwise correlations between individual traits of *Bunias orientalis* plants
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